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Candida: A Causative Agent of an Emerging Infection

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Incidences of infections due to *Candida* have increased over the last 15–20 y. This increase in the incidence and the high associated mortality rate despite therapy has focused the attention on this disease and prompted investigators to undertake research aimed at understanding the pathogenesis of this disease as well as methods to treat it. This paper discusses recent developments in the *Candida* field and the impact they have on patient management. ***Key words: antifungals management/diagnosis/susceptibility. Journal of Investigative Dermatology Symposium Proceedings 6:188–196, 2001***

Emerging infectious diseases are diseases of infectious origin whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future. In this regard, *Candida* is among those diseases whose incidence has increased over the past 15–20 y. Wey *et al* (1998) provided the first evidence that fungi had begun preying on the growing population of patients with impaired immune systems (e.g., AIDS, cancer patients). In their study, these authors showed that nearly 40% of all deaths from hospital-acquired infections were not due to bacteria or viruses, but to fungi, with the main culprit being *Candida*. Supporting evidence for the increase in the incidence of candidal infections could be derived from various databases, including the National Hospital Discharge Survey (NHDS), the Professional Activity Survey (PAS), and the McDonnell Douglas Automation Company Medical Records System (McAuto), which showed that in the 1980s the incidence of candidiasis in hospitalized patients has increased from <0.5 to about 2 per 1000 hospitalized admission (Fisher-Hoch and Hutwagner, 1995). Interestingly, age-specific rates of oropharyngeal candidiasis derived from NHDS data demonstrated clearly that the increase in this incidence was true irrespective of the age group (Fisher-Hoch and Hutwagner, 1995). Follow-up surveillance studies conducted in the 1990s demonstrated that the high incidence of candidal infections in hospitalized patients is still true. Edmond *et al* (1999) undertook a survey referred to as Scope Project (Surveillance and Control of Pathogens of Epidemiologic Importance) where they conducted concurrent surveillance for bloodstream infections at 49 hospitals over a 3 y period. In this study, more than 10 000 Gram-positive, Gram-negative, and yeast infections were monitored. Data analyzes showed that whereas Gram-positive and Gram-negative bacteria accounted for 64% and 27% of cases, respectively, yeast accounted for 8% of cases. Furthermore, *Enterobacteria*, *Serratia*, and coagulase (–) staphylococci and *Candida* were more likely to cause infection in the critical care setting. This study shows that *Candida* continues to be an important cause of nosocomial bloodstream infection. Importantly, similar to earlier findings, yeasts were associated with the highest crude mortality (40%). Unlike the situation with candidal bloodstream infections, which remain high, the incidence of esophageal candidiasis in the HIV/AIDS setting has decreased. This decrease could be attributed to the host immune reconstitution brought about by the new antiretroviral therapy (Highly Active Antiretroviral Therapy, HAART). The decrease in esophageal candidiasis in both adults and children is documented in the study of Kaplan *et al* (2000), who investigated the epidemiology of HIV-associated opportunistic infections (OI) in the U.S.A. in the era of HAART (Kaplan *et al* 2000). These authors showed that, among other OI, the incidence of esophageal candidiasis has dropped significantly between 1996 and 1997; however, a disturbing trend is seen between 1997 and 1998, where the incidence of esophageal candidiasis seems to be on the incline again. This observation indicates that it is prudent to continue following the epidemiology of this and other opportunistic infections, and that it is too early to draw firm conclusions regarding such trends. The decrease in the incidence of OI, including candidiasis, has been attributed to the restoration of specific immunity (Kelleher *et al*, 1996; Autran *et al*, 1997; Pakker *et al*, 1997; Angel *et al*, 1998); however, protease inhibitors could also be exerting a direct effect on *C. albicans* secretory aspartyl proteases (Sap), which have been shown to be virulence factors for *C. albicans* (Hube, 1996). In this regard, Cassone *et al* showed that the protease inhibitors indinavir and ritonavir inhibited Sap activity and production (Cassone *et al*, 1999). Further studies are required to demonstrate whether Sap inhibition is implicated in the decrease of candidiasis in AIDS patients. It is critical to clarify that the observation that the incidence of OI, including candidiasis, is decreasing in the HIV-infected patients is limited to developed countries (mainly the U.S.A. and Western Europe). The incidence of these devastating diseases is still high in developing countries, however, particularly sub-Saharan Africa where between 20% and 50% (depending on the country or regions within a country) of the population are infected with this virus (Schooley, 2000).

The increase in the incidence of serious candidiasis and the high associated mortality rate, even with therapy (35%–50%), has focussed the attention on this disease and prompted investigations aimed at improving our understanding of the pathogenesis of this disease, and host–parasite interactions (Pittet *et al*, 1994). This paper will focus on

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recent developments in the *Candida* field that impact patient management.

RISK FACTORS AND *CANDIDA SPECIES*

Candidiasis is an infection of the immunocompromised. This implies that for *Candida* to cause an infection, one or more risk factors should be present. A number of factors have been suggested, including underlying immune insufficiency, use of broad-spectrum antibiotics, cytotoxic agents (e.g., anticancer agents), immunosuppressive drugs, and central venous catheters.

Although a number of *Candida* species exist, only 10 species have been associated with infection to any significant degree. These include: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. guilliermondii*, *C. pseudotropicalis*, *C. rugosa*, and *C. stellatoidea*. For a long time (up to early 1990s), *C. albicans* was the main etiologic agent of candidiasis, responsible for more than 70% of all *Candida* infections. With the introduction of the azoles (late 1980s, early 1990s), a shift in the candidal species causing infection started to occur with non-*albicans* becoming more important in the disease process. This point is clearly shown in the Edmond *et al* study that reported that the non-*albicans* species caused between 30% and 54% of the yeast infection (Edmond *et al*, 1999). Moreover, these authors showed that the extent of yeast infections caused by non-*albicans Candida* varied with the geographic region. According to their study, the north-east region of the U.S.A. had the highest percentage (54%) of nosocomial bloodstream infections due to non-*albicans* species, and the south-west had the least (30%). *Candida glabrata* and *C. tropicalis* were the second and third causative *Candida*, after *C. albicans*, respectively (Edmond *et al*, 1999). This change in the pattern of candidal species causing infections impacts the management of this disease. For example, *C. glabrata* tends to have higher minimum inhibitory concentrations (MIC) values to the currently used antifungals, particularly the azoles (MIC 16–32 μg per ml). Moreover, infections caused by this species are associated with poor prognosis (Nguyen *et al*, 1996). Thus, the use of high doses of fluconazole (800 mg daily) is recommended for the management of candidiasis caused by *C. glabrata*.

DIAGNOSIS

Currently, bedside evaluation, blood cultures, diagnostic imaging, and biopsies are standard clinical and laboratory tools used in the diagnosis of invasive candidiasis. Unfortunately, these methods lack sensitivity in the early recognition of this infection and are imprecise as markers of complete eradication of infection. Many of the early attempts of nonculture diagnosis of invasive candidiasis lack sensitivity; however, advances in the identification and purification of potential antigens, monoclonal antibody production, epitope mapping, recombinant DNA techniques, and polymerase chain reaction (PCR) should contribute towards further development of diagnostic methods for fungi that are increased in both sensitivity and specificity. As these studies have been extensively reviewed (Jones, 1990; Walsh *et al*, 1991; de Repentigny, 1992; Reiss and Morrison, 1993; Verweij *et al*, 1998), a brief description of the current status of research will be provided in this proposal.

Culture-based methods Detection of *Candida* spp. in the bloodstream is a key factor for establishing a diagnosis of invasive candidiasis. There have been numerous advances in blood culture methodology over the past 10–15 y that have contributed to improved detection of candidemia (Bougnoux *et al*, 1990; Kohno *et al*, 1993; Chen *et al*, 1997). Presently the lysis centrifugation and Bactec methods are the most superior of the systems developed. Although these methods are clearly a technologic advance these systems have a low sensitivity in the early detection of candidemia (between 25% and 82%) (Jones, 1990). Additionally, analysis of the available data indicates that maximum detection of fungemia is achieved when more than one blood culture system is employed (Pfaller and Wenzel, 1992). The length of time (minimum of 5 d) needed to recover *Candida* is another limitation with blood culture methods. These drawbacks emphasize the need for innovative nonculture methods that allow early detection of candidiasis, and/or complement blood culture.

Non-culture methods Molecular and biochemical factors involved in *Candida* infections have been investigated to develop nonculture diagnostic methods. These factors include (i) *Candida* antigens, (ii) antibodies against *Candida* antigens, (iii) *Candida* metabolites, and (iv) *Candida* DNA sequences.

Detection of *Candida* antigens Several workers attempted to develop immunoassays for detecting *C. albicans* antigens in sera from infected patients (Bennet, 1987; Jones, 1990; de Repentigny, 1992; Reiss and Morrison, 1993). These investigators employed radioimmunoassay (RIA), enzyme-linked immunoassay (ELISA), and latex agglutination to identify circulating and/or cytoplasmic antigens.

Cell-wall mannans The cell wall mannan (mannoprotein) of *Candida* has been studied extensively at the immunologic level. Thus, mannan extracts of varying degrees of purity have been used for serodiagnosis (Odds, 1988). Cell wall mannan antigen is not usually detectable unless the immune and other circulating complexes are disrupted by hydrolysis (Walsh *et al*, 1995). Another limitation to using mannan is that it is cleared rapidly from the serum (i.e., transient). Consequently, repeated serum sampling is necessary (Jones, 1990; de Repentigny, 1992). In spite of these limitations a number of commercial tests are available in Europe for mannan detection. Unfortunately the sensitivity of these tests is still a drawback (Fujita and Hashimoto, 1992).

Enolase *Candida* enolase, a 48 kDa cytoplasmic antigen, is recognized by human sera, and is associated with invasive candidiasis (Strockbine *et al*, 1984). A multicenter study of high-risk cancer patients with invasive candidiasis showed that the enolase-based method has a 54% sensitivity (Walsh *et al*, 1991). Multiple serum sampling improved sensitivity (up to 75%) and specificity (up to 96%). Although enolase showed a lot of initial promise, developing a commercial assay was not cost effective (Sendid *et al*, 1999). Furthermore, the need for multiple sampling to improve sensitivity indicates that enolase-based diagnostic assay can complement but not replace blood cultures in the diagnosis of invasive candidiasis (Walsh *et al*, 1991).

Detection of circulating antibodies Anti-*Candida* antibodies have been investigated as diagnostic markers of invasive candidiasis (Taschdjian *et al*, 1973; Kozinn *et al*, 1978; van Deventer *et al*, 1996). Anti-*Candida*-enolase antibody was detected in sera of less severely immunocompromised patients with invasive candidiasis (Walsh *et al*, 1991). Furthermore, antimannan antibody was detected in patients with mycologically and clinically proven candidiasis (Sendid *et al*, 1999). Unfortunately, the low, sometimes undetectable, levels of certain antibodies in immunocompromised patients limit their reliability as diagnostic markers.

Detection of *Candida* metabolites

D-arabinitol D-arabinitol, a sugar alcohol metabolite from *C. albicans*, has been used as a marker for invasive candidiasis (Christensson *et al*, 1997). D-arabinitol is cleared by glomerular filtration in the body. Thus, impaired renal function may lead to elevated serum concentrations of D-arabinitol, which can be detected by gas-liquid chromatography (GLC) or by mass spectroscopy (MS) with selected ion monitoring. Elevated levels of D-arabinitol are observed in cases of invasive candidiasis, but not in infections due to *C. glabrata* and *C. krusei* (Lehtonen *et al*, 1996). Although elevated levels of D-arabinitol are specific to invasive candidiasis, the amount of time and cost involved and also the complexities of GLC and MS discourage the use of this method as a routine diagnostic tool (McSharry *et al*, 1993). Nonetheless, an enzymatic-fluorometric method for determination of serum D-arabinitol has been developed using *Klebsiella pneumoniae*

D-arabinitol dehydrogenase that oxidizes D-arabinitol (Walsh *et al*, 1994). The utility of D-arabinitol is still under investigation.

1,3-β-D-glucan 1,3-β-D-glucan is a major structural component of the fungal cell wall that is released by several pathogenic fungi including *C. albicans*. Detection of 1,3-β-D-glucan is based on the coagulation system in the *Limulus* amoebocyte (Ikegami *et al*, 1988; Obayashi *et al*, 1995). Although 1,3-β-D-glucan has been detected in plasma from patients with systemic mycoses, concentration of this component was significantly higher in those with invasive candidiasis. Thus, this assay may have some clinical utility for diagnosis of invasive candidiasis; however, this approach is still under investigation.

Amplification of *Candida* DNA by PCR Several laboratories are actively pursuing the development of PCR-based tests for rapid diagnosis of invasive candidiasis. These assays have focussed on the use of universal fungal primers, multicopy gene targets, and species-specific DNA regions (Reiss *et al*, 1998). The universal fungal oligonucleotide primer pair ITS86 and ITS4, which are designed based on the conserved sequences of the 5.8S and 28S ribosomal DNA, respectively, amplifies a species-specific internal transcribed spacer, ITS2 region (Lott *et al*, 1998). The variability in length of this region between different fungal species is the basis for diagnosis. Similarly, sequences from the fungal lanosterol 14α-demethylase gene have been used in a PCR approach to develop a diagnostic test for invasive candidiasis (Buchman *et al*, 1990; Morace *et al*, 1999). The family of secreted aspartic proteinase genes has also been the focus of PCR amplification (Flahaut *et al*, 1998).

The use of PCR as a diagnostic technique for fungal infections has gained favor because it is rapid, sensitive, and specific; however, the high sensitivity can in fact be a potential disadvantage because detection of commensal *C. albicans* strains, in otherwise uninfected individuals, may produce false positive reactions (Flahaut *et al*, 1998). Likewise DNA sequences from dead or degrading fungal cells may also be amplified.

Taken together, although extensive attempts at developing methods to diagnose fungal infections, including *Candida*, are currently being pursued, all of these methods are still investigational, making conclusions regarding their efficiency premature. To date, the contributions of antibody or antigen detection tests remain unsatisfactory. Studies using PCR to detect candidiasis in neutropenic patients have shown some promise; however, clinical trials are needed to evaluate their utility.

ANTIFUNGAL SUSCEPTIBILITY TESTING AND RESISTANCE

The development of standardized antifungal susceptibility testing procedures was spearheaded by the National Committee for Clinical Laboratory Standards (NCCLS), Subcommittee for Antifungal Susceptibility Testing. This development passed through a number of phases:

(i) Examination of the role of variables such as inoculum preparation, inoculum size, medium composition, incubation temperature, incubation time, and endpoint definition on interlaboratory variability (Guinet *et al*, 1988; Pfaller *et al*, 1988, 1990; Espinel-Ingroff *et al*, 1991, 1992; Shawar *et al*, 1992; Fromtling *et al*, 1993; Troillet *et al*, 1993; Barchiesi *et al*, 1995; Ghannoum *et al*, 1996).[1]

(ii) Publication of the NCCLS document M27-p (proposed), which set the initial conditions for the standardized method: a macrobroth methodology using a starting yeast inoculum of 0.5–2.5 × 10^3 cells per ml, RPMI-1640 medium buffered to pH 7.0, incubation for 48 h (*Candida* species) or 72 h (*Cryptococcus neoformans*) at 35°C and carefully defined endpoint criteria.

(iii) Revision and publication of the M27-T as a tentative standard. The main changes in this version included a microdilution format for the standard method, modifications on determining the susceptibility testing of *C. neoformans*, and determining the susceptibility of *Candida* against amphotericin B. The second addition to the M27-T document addressed the difficulty inherent in determining the susceptibility of yeast to amphotericin B using RPMI-1640. This medium tends to cluster the MIC values in a very narrow range, making it difficult to differentiate between resistant and susceptible isolates (Ghannoum *et al*, 1992; Rex *et al*, 1995).

(iv) Revision and publication of M27-A (approved) (National Committee for Clinical Laboratory Standards, 1997). This represents the final stage in the development of the standardized method for antifungal susceptibility methodology for yeast. The main modification introduced in this document is the development of breakpoints for antifungal susceptibility testing (see below for details).

Now that an approved version of M27 is published, it is important to stress that the process of developing standardized susceptibility testing for fungi is a dynamic process; and a number of studies are currently underway to make the yeast method more amenable for routine clinical use (Rex *et al*, 1995; Tiballi *et al*, 1995; Hawser *et al*, 1998; Lozano-Chiu *et al*, 1998). Moreover, a number of investigations are currently underway to develop optimal methods for determining the antifungal susceptibility testing of dermatophytic fungi. Although this topic is beyond the scope of this review, interested readers should consult Norris *et al* (1999) and Jessup *et al* (2000a).

Clinical utility of the antifungal susceptibility testing method For a susceptibility testing method to be clinically useful, it should have the ability to predict clinical outcome. A number of studies were performed to test whether antifungal susceptibility testing methods, including NCCLS M27, can be used to predict outcome. These investigations have been previously reviewed and will not be presented here in detail (see Ghannoum *et al*, 1996; Reyes and Ghannoum 2000). Briefly, these reports show that the largest number of studies correlating *in vitro* antifungal susceptibility testing with clinical outcome involve fluconazole, and focus on oropharyngeal candidiasis in patients with AIDS (Horsburgh and Kirkpatrick, 1983; Merz, 1984; Wingard *et al*, 1991; Ghannoum *et al*, 1992; Reynes *et al*, 1992; Bart-Delabesse *et al*, 1993; Pfaller *et al*, 1994; Sangeorzan *et al*, 1994; Ghannoum, 1996).[2]

To determine whether or not a correlation exists between MIC data and clinical outcome, the NCCLS Subcommittee for Antifungal Susceptibility Testing reviewed data on fluconazole and itraconazole, provided by Pfizer Pharmaceuticals and Janssen Pharmaceuticals, respectively, and proposed tentative breakpoints for MIC for these drugs as determined by the NCCLS M27 macrobroth methodology. Analysis of these data showed strong correlation between MIC and clinical outcome. For fluconazole patients on a dose of 100 mg per day, the success rate was >95% up to an MIC of 8 μg per ml. Conversely, the success rate for isolates with MIC ⩾ 16 μg per ml was lower with an overall success rate of 76%. The correlation between MIC data and clinical correlation becomes even more obvious when we consider isolates obtained from patients treated with more than 100 mg per day of fluconazole. The success rate in this group is strikingly uniform at 80%–90% for all isolates with MIC ⩽ 32 μg per ml. A significant decrease in the success rate is, however, noted for the isolates with MIC of 64 μg per ml and greater (46% success rate). Thus the data support the concept that response to fluconazole is a function of both MIC and drug dose.

Since 98% of candidal strains used for analysis of *in vitro–in vivo* correlation in patients treated with itraconazole were obtained from

[1]Troke PF: Fluconazole Susceptibility Testing Group. Standardized susceptibility testing of fluconazole: a nine centre, international collaborative study. In: *Abstract 32nd Interscience Conf Antimicrob Agents Chemother.* Washington, DC: American Society for Microbiology, 1992, Abstract 1596

[2]Ganger G, Just-Nubling G, Eichel M, Hoika R, Stille W, Goethe J: Itraconazole solution in patients with non-response to fluconazole. In: *Abstract 9th Int Conf on AIDS.* Berlin: International AIDS Society, PO-B09-1394, 1993

Table I. Tentative interpretive guidelines for susceptibility testing *in vitro* of *Candida* species

Antifungal agent	Susceptible (S)	Susceptible-dose dependent (S-DD)[a]	Resistant
Fluconazole	⩽ 8	16–32	⩾ 64
Itraconazole	⩽ 0.125	0.25–0.5	⩾ 1
5-fluorocytosine	⩽ 4	8–16	⩾ 16

[a]For 5-fluorocytosine, the old term "intermediate susceptibility" (I) is used instead of S-DD by the NCCLS Committee. Values are in μg per ml.

individuals treated with a 200 mg per day dose, dosage was not considered in the analysis; however, itraconazole plasma levels were available for many of the patients. Furthermore, data in the literature indicate that patients with AIDS who are infected with candidal isolates and who have decreased susceptibility to fluconazole, may respond to higher doses of itraconazole (Fromtling *et al*, 1993; Warnock *et al*, 1999). Therefore, the committee decided to include plasma levels in the analysis. Examination of the data shows that the success rate of itraconazole is ⩾ 81% for those patients infected with isolates with an MIC of ⩽ 0.125 μg per ml and the outcome appears to be independent of drug plasma levels. Among patients with itraconazole plasma levels ⩽ 5 μg per ml, the success rates decrease for those individuals infected with isolates inhibited by 0.25–0.5 μg per ml and ⩾ 1.0 μg per ml (50% and 44% success rates, respectively). This correlation is not as well defined for patients with plasma levels > 0.5 μg per ml. Overall, the analysis suggests that although infections due to isolates with itraconazole MIC ⩾ 0.25 μg per ml are less likely to respond than those due to more susceptible organisms, the therapeutic effect may be enhanced by ensuring that the plasma levels exceed 0.5 μg per ml. It is worth mentioning that the data analyzed for itraconazole are exclusively for patients with oropharyngeal candidiasis.

Based on the analysis of the data packages discussed above, the NCCLS proposed tentative breakpoints for antifungal susceptibility testing of fluconazole and itraconazole. The breakpoints and the conceptual framework followed to develop them are elegantly presented by Rex *et al* (1997). **Table I** summarizes the breakpoints to these agents. Interpretive breakpoints for 5-fluorocytosine were also proposed by the NCCLS Committee. As data similar to that of fluconazole and itraconazole were not available to the Committee, the proposed breakpoints for 5-fluorocytosine were based on historical data. Finally, interpretive breakpoints for amphotericin B were not put forward. This could be attributed primarily to the fact that M27 methodology does not discriminate between susceptible and resistant isolates. Thus, amphotericin B breakpoints must await further studies that will allow such discrimination.

Now that the interpretive breakpoints have been in use for the last 3 y, it is clear that physicians are using them with some degree of success. This is evident by the recommendation of the Mycoses Study Group to use antifungal susceptibility testing in the management of patients with candidemia and acute hematogenously disseminated candidiasis (Rex *et al*, 1995). Having said that, it is important to put the predictive power of antifungal susceptibility testing in perspective. Although good to excellent statistical correlations have been shown between treatment success and MIC values, physicians should consider other factors prior to institution of treatment. Fungal infections are diseases of the immunocompromised, and all too often underlying diseases and other iatrogenic factors, unrelated to MIC, may be the deciding factor in success or failure of a given treatment.

ANTIFUNGAL AGENTS IN CLINICAL USE

Polyenes Since its discovery in the 1950s, amphotericin B has been the most widely used antifungal agent to treat invasive or life-threatening fungal infections. Amphotericin B has fungicidal action against microorganisms that contain sterols (ergosterol in particular) in their outer cell membrane. The interaction of amphotericin B with membrane sterol results in the production of aqueous pores containing an annulus of eight amphotericin B molecules linked hydrophobically to the membrane sterol (Holz, 1974). This configuration gives rise to a pore in which the polyene hydroxyl residue faces inward, leading to altered permeability, leakage of vital cytoplasmic components, and death of the organism (Kerridge, 1980). Despite the broad-spectrum of fungicidal activity, amphotericin B has a narrow therapeutic index that limits its clinical usefulness (Gallis *et al*, 1990). Fever, chills, nausea, vomiting, headache, and electrolyte imbalance are common; thrombocytopenia, generalized seizure, and shock are also reported infrequently in patients treated with amphotericin B. In children, overdose has been found to cause arrhythmia, cardiac arrest, or even death (Cleary *et al*, 1993). The exact mechanism for toxicity of amphotericin B is not clear, but nephrotoxicity may arbitrarily be classified as vascular or tubular, and the interference of amphotericin B with mammalian membrane properties may underlie all of the nephrotoxic effects (Sawaya *et al*, 1995). Several strategies including combination therapy, modification of amphotericin B molecule and of the physical state of amphotericin B, or changes in drug delivery system have been used to overcome the toxic side-effects of amphotericin B (Hossain and Ghannoum, 2000). Incorporation of amphotericin B in lipid represents one such approach. The lipid formulations of amphotericin B, complexed with phospholipid or detergent, can modify the distribution of amphotericin B *in vivo*, maintaining activity and lowering toxicity (Brajtburg and Bolard, 1996).

Three types of lipid formulations of amphotericin B, namely amphotericin B lipid complex (abelcet), amphotericin B colloidal dispersion (amphotec), and liposomal amphotericin B (amBisome), incorporated into sheet, disc, or liposome, respectively, are available for clinical use. In the U.S.A., abelcet, amphotec, and amBisome are being used for treating invasive fungal infections in patients intolerant or refractory to conventional amphotericin B. AmBisome is also being used for empiric treatment of febrile neutropenic patients. The three lipid formulations are effective against pathogenic fungi, and are less toxic than conventional amphotericin B. Efficacy data available on the new products cover a wide variety of systemic mycoses including candidiasis (Wong-Beringer *et al*, 1998).

Azoles In view of the toxicity of amphotericin B and the limited activity and development of resistance to flucytosine, discovery of the azoles in 1970s was considered a major therapeutic breakthrough in antifungal chemotherapy. The approval of the imidazoles and the triazoles (the N-substituted imidazoles) in the late 1980s and the early 1990s was a major advancement in safe and effective treatment of superficial and deep fungal infections. These compounds exert fungistatic effects by inhibition of sterol biosynthesis. The primary target of azoles is the heme protein, which cocatalyzes cytochrome P450-dependent 14α-demethylation of lanosterol (Hitchcock *et al*, 1990). Inhibition of 14α-demethylase leads to the depletion of ergosterol, the bioregulator of membrane fluidity and asymmetry, and to the accumulation of sterol-precursors including 14α-methylated

sterols. These membranes fail to maintain optimum permeability with a consequent loss of essential cytoplasmic material and eventual cell death.

The clinical efficacy and safety of the second-generation azoles, fluconazole followed by itraconazole in particular, have had a dramatic impact on the management of invasive fungal infections caused by a wide variety of fungi, including yeasts, dermatophytes, and to some extent molds. Itraconazole and fluconazole have become frequently used therapeutic alternatives to amphotericin B (Kauffman and Carver, 1997); however, with the widespread use, resistance to azoles has emerged and become of some concern. *Candida albicans* strains from AIDS patients with oropharyngeal candidiasis often show resistance to fluconazole and, in some strains, cross-resistance to itraconazole and ketoconazole (Laguna *et al*, 1997). Prolonged itraconazole prophylaxis in AIDS patients has also been found to result in reduced susceptibility to itraconazole and cross-resistance to fluconazole (Goldman *et al*, 2000). Extensive studies on azole-resistance mechanisms in *C. albicans* strains have been done. Of particular importance are enhanced activity of cytochrome P450 14α-demethylase enzyme (Vanden Bossche *et al*, 1994), energy-dependent efflux pump (Prasad *et al*, 1995), and decreased susceptibility to azoles (Lamb *et al*, 1997). Various mechanisms have also been proposed for resistance in *C. glabrata* strains, such as energy-dependent drug efflux (Miyazaki *et al*, 1998) and deficiency of mitochondrial DNA (Defontaine *et al*, 1999). Irrespective of the mechanisms involved in azole-resistance there is a significant impact on treatment outcome. For a review of the mechanisms of antifungal resistance, the reader is referred to the recent article by Ghannoum and Rice (1999). Another potential limitation of the azole antifungal drugs (particularly itraconazole) is the frequent drug interactions that result in adverse clinical consequences, such as lowered potency of the azole or toxicity of the coadministered drug (Hoesley and Dismukes, 1997).

Pyrimidine analog Antifungal activity of flucytosine (5-fluorocytosine, 5-FC) was reported in the early 1960s, although the only drug of the class was approved for use in humans in the early 1970s. 5-FC is a fluorinated pyrimidine with inhibitory activity against pathogenic fungi, including *Candida* species and *Cryptococcus neoformans*. 5-FC enters the fungal cells aided by a permease enzyme. Inside the cell, it is converted to 5-fluorouracil (5-FU) by the enzyme cytosine deaminase. Subsequently, 5-FU is converted by UMP pyrophosphorylase into 5-fluorouridylic acid, which is further phosphorylated and incorporated into RNA, resulting in disruption of protein synthesis (Polak and Scholer, 1975). 5-FU is also converted to 5-fluorodeoxyuridine monophosphate, a potent inhibitor of thymidine synthase, an enzyme involved in DNA synthesis and nuclear division (Diasio *et al*, 1978). Thus, 5-FC acts by interfering with pyrimidine metabolism, as well as RNA, DNA, and protein synthesis in fungal cells. The drug has a narrow spectrum of activity and 5-FC monotherapy is not encouraged because of easy development of resistance. Thus, it is usually administered in combination with amphotericin B or fluconazole to treat systemic mycoses. In addition to gastrointestinal upset including nausea, vomiting, and anorexia, as well as bone marrow suppression, 5-FC may cause impairment of renal function when administered with a nephrotoxic drug such as amphotericin B. Thus, monitoring of the serum level and rational dose adjustment may be needed for a successful treatment outcome.

Newer antifungal agents

New formulation of polyene compounds Nystatin, the first polyene antifungal agent discovered in the 1950s, has been a well-known drug for topical administration. Nystatin, being structurally related to amphotericin B (tetraene macrolide), acts to some extent the same way. Despite its fungicidal property, nystatin has not been developed for treatment of systemic fungal infections because of its significant nephrotoxicity. Nystatin is an excellent drug for treatment of superficial *Candida* infections, but is not effective against dermatophyte infections.

Liposomal nystatin, Nyotran, a liposomal formulation of nystatin being developed by Aronex Pharmaceuticals, prepared by incorporation of nystatin into multilamellar liposome, was found to retain the *in vitro* antifungal activity of conventional nystatin and protect human erythrocytes from lysis, suggesting lower toxicity.[3,4] Unlike the free nystatin that was toxic and lethal, liposomal nystatin at an equivalent concentration was well tolerated. Improved survival of mice and improved *in vivo* activity at higher doses prompted clinical trials (Mehta *et al*, 1987). In a rabbit model of subacute disseminated candidiasis, multilamellar liposomal nystatin was found to be less nephrotoxic and had concentration-dependent activity comparable with that of amphotericin B or fluconazole (Groll *et al*, 1999). Nyotran is currently undergoing clinical trials.

Triazoles with extended spectrum of activity The therapeutic success of fluconazole encouraged the development of new triazole antifungal agents. The new third-generation azoles are characterized by their broad spectrum activity and low toxicity. The new triazoles share similar mechanisms of action as earlier triazoles, and demonstrate potent antifungal activity against both fluconazole-susceptible and -resistant organisms. In this paper, a brief discussion of three triazoles (voriconazole, posaconazole, and ravuconazole) will be presented.

Voriconazole (UK-109 496), a triazole developed by Pfizer, is the most advanced in its preclinical and clinical evaluation. Voriconazole is structurally related to fluconazole. It is available in oral and intravenous formulations. Voriconazole was 8- to 130-fold more active than fluconazole against *Candida* species sensitive or resistant to fluconazole, including *C. glabrata* and *C. krusei*. It appears to function by inhibiting fungal P450-dependent 14α-demethylase enzyme, blocking sterol biosynthesis in a concentration-dependent manner (Belanger *et al*, 1997). Treatment of fluconazole-susceptible and -resistant *C. albicans* with voriconazole completely blocked ergosterol synthesis and caused a significant increase in the level of squalene, 4,14-dimethylzymosterol, 24-methylenedihydrolanosterol, and zymosterol. Accumulation of 24-methylenedihydrolanosterol was observed in *C. albicans* and *C. krusei*. Accumulation of the methylated sterols is consistent with the premise that voriconazole inhibits fungal growth by interfering with cytochrome P450-dependent 14-α-demethylase, a known target enzyme for azoles. In a comparative study, voriconazole showed potent *in vitro* concentration-dependent antifungal activity, as well as more activity than fluconazole against several clinical strains of *C. glabrata* (Koul *et al*, 1999). In another study, voriconazole showed *in vitro* activity against both fluconazole-susceptible and -resistant *Candida* species and was more potent than itraconazole and ketoconazole (Nguyen and Yu, 1998). Voriconazole was found to inhibit the growth and alter the morphology of these candidal strains. Exposure of yeast to voriconazole caused growth inhibition, cell wall thinning, and cell membrane degradation (Sanati *et al*, 1997). Voriconazole was more active than fluconazole in the inhibition of putative virulence factors such as endothelial cell adherence, germination, and endothelial cell injury by both fluconazole-resistant and fluconazole-sensitive strains of *C. albicans*. It was also able to interfere with endothelial cell damage by *C. krusei* (Fratti *et al*, 1998). Phase III clinical trials with voriconazole have been completed.

[3]Wallace T, The Nyotran Study Group: *In vitro* antifungal activity of nyotran against clinical isolates. *Abstracts of the 14th Conference of the International Society for Human and Animal Mycology. Buenos Aires, Argentina, 2000*, abstr. 408

[4]Jessup C, Wallace T, Ghannoum MA: An *in vitro* interaction study with Nyotran (liposomal Nystatin) and conventional antifungals, antibiotics, antivirals, and immunosuppressive drugs against common fungal pathogens. *Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. San Francisco: American Society for Microbiology, 1999, abstr. 163

Posaconazole (SCH 56592) is a new broad-spectrum triazole that is being developed by Schering-Plough. Posaconazole is a hydrogenated analog of itraconazole with a 1,3-dioxolone backbone. It is a potent inhibitor of lanosterol 14-α-demethylase and has shown *in vitro* and *in vivo* activity against a variety of pathogenic fungi, including *Candida* spp. (Law *et al*, 1997), *C. neoformans* (Perfect *et al*, 1996), *A. fumigatus* (Oakley *et al*, 1997), *B. dermatitidis* (Sugar and Liu, 1996), *C. immitis* (Lutz *et al*, 1997), and *H. capsulatum* (Connolly *et al*, 1999). In a comparative *in vitro* susceptibility study, posaconazole was more active than fluconazole and slightly more active than either itraconazole or ketoconazole against a large number of clinical yeast isolate (Barchiesi *et al*, 2000).

Ravuconazole (BMS-207147, formerly ER-30346) originally discovered by Eisai (Japan), and licensed by Bristol-Myers Squibb, is a new investigational triazole antifungal agent. Broad-spectrum activity has been demonstrated for ravuconazole against various pathogenic fungi (Fung-Tomc *et al*, 1998). A large-scale comparative *in vitro* study showed ravuconazole was as active as posaconazole or voriconazole against all clinical isolates of *Candida* species, and more active than fluconazole and itraconazole; however, ravuconazole, posaconazole, and itraconazole were more active than fluconazole but less active than voriconazole against *C. glabrata* (Pfaller *et al*, 1998a). MIC values of ravuconazole were lower than those of itraconazole against *Candida* species, including fluconazole-resistant *C. albicans*, *C. glabrata*, and *C. krusei*. In addition, ravuconazole was minimally affected by overexpression of the gene encoding efflux pump *MDR1* (Fung-Tomc *et al*, 1999).

Antifungals targeting fungal cell wall Fungal cell walls are composed of some unique components such as (1,3)-β-D-glucan, mannan, and chitin. Unlike sterols, these components have no mammalian counterpart. This uniqueness provides the advantage of selective toxicity. Thus, polysaccharides are considered as novel targets for antifungal therapy. The cell wall of most pathogenic fungi, including *C. albicans*, is a multilayered structure, with its outer layer composed of mannan, mannoprotein, and (1,6)-β-glucan; the inner layer is composed predominantly of (1,3)-β-glucan, chitin, and some mannoprotein (Surarit *et al*, 1988). (1,3)-β-D-glucan is one of the essential structural components of fungal cell walls, and has been used as a marker in serodiagnosis of invasive fungal infection (Hossain *et al*, 1997; Yoshida *et al*, 1997).

Echinocandins and closely related pneumocandins are amphophilic lipopeptides that target (1,3)-β-D-glucan. These compounds act by specific and noncompetitive inhibition of the (1,3)-β-D-glucan synthase enzyme complex (which is essential for formation of glucan polymers of the fungal cell wall), leading to depletion of cell-wall glucan, osmotic instability, and lysis of fungal cell (Debono and Gordee, 1994). Considering their novel mode of action, these semisynthetic cyclic lipopeptides are being tested as parenteral or oral agents for their antifungal potential. Of the three groups of compounds (echinocandins, papulocandins, and aculeacin) that comprise the novel class of fungal lipopeptides, only echinocandins have been extensively evaluated for their safety, tolerability, and efficacy against *C. albicans* and other fungi. Unlike cilofungin, an early echinocandin that was not developed because of narrow spectrum of activity and nephrotoxicity, more improved derivatives of echinocandins have been developed recently.

Caspofungin (MK-0991, formerly L 743872), being developed by Merck, is a water-soluble antifungal with fungicidal activity against yeasts in particular. Caspofungin has shown *in vitro* activity against clinical isolates of filamentous fungi, including *Aspergillus*, *Fusarium*, *Rhizopus*, and *Pseudallescheria* species (Pfaller *et al*, 1998b). Caspofungin also showed *in vitro* activity against medically important yeasts, and had low hemolytic potential (Bartizal *et al*, 1997). Caspofungin is undergoing phase II and III clinical trials.

FK 463 is an echinocandin-like compound developed by Fujisawa. It is a water soluble, semisynthetic lipopeptide isolated from the culture broth of *Coleophoma empedri* and is suitable for intravenous use. It is a fungicidal agent, and is effective against *C. albicans*, *C. tropicalis*, and *C. glabrata* and is more potent than amphotericin B; however, it is fungistatic against *Aspergillus* species, less active against *C. krusei*, *C. parapsilosis*, and *C. guilliermondii*, and inactive against *C. neoformans*, *Fusarium solani*, *Trichosporon asahii*, and *T. cutaneum* (Tawara *et al*, 2000). MIC of FK 463 were found to be lower than those of several azoles and conventional amphotericin B against azole-resistant *CDR*-expressed and *CaMDR*-expressed *C. albicans* strains. In addition, in a murine model of hematogenously disseminated candidiasis, FK 463 showed efficacy against azole-resistant *C. albicans* strains that led to 100% survival, significant reduction of the tissue fungal burden, and the lowering of the concentration of (1,3)-β-D-glucan in plasma. The efficacy of FK 463 was superior to that of amphotericin B and fluconazole (Maesaki *et al*, 2000). Thus, FK 463 has a potential for treatment of disseminated candidiasis caused by azole-resistant strains.

MANAGEMENT OF CANDIDIASIS

As *Candida* causes a wide range of infections, ranging from nonlife-threatening mucocutaneous infections to invasive processes that may involve virtually any tissue, management of the different clinical manifestations of candidiasis requires a broad range of therapeutic and diagnostic strategies (Rex *et al*, 2000). These strategies were recently addressed in two publications: (i) ''International Conference for the development of a consensus on the management and prevention of severe candidal infections'' (Edwards *et al*, 1997) and (ii) ''Practical guidelines for the treatment of candidiasis'' (Rex *et al*, 2000). For an in-depth coverage of this issue, the reader should consult these two seminal articles. Both documents stress that there is no one strategy to follow to manage patients with candidiasis. In this regard, the choice of therapy depends on the clinical status of the patient, the physician's knowledge of the species, and the antifungal susceptibility of the infecting isolate. The general strategy for the management of candidemia and acute hematogenously disseminated candidiasis entails that all patients be treated. It is recommended that patients with candidemia be treated for 2 wk following the last positive culture. Moreover, patients begun on amphotericin B may be switched to fluconazole to complete the course of therapy. It is imperative that all existing central venous catheters be removed. Finally, it is critical that patients with suspected disseminated candidiasis undergo ophthalmologic examination. Failure to do so often has grave consequences such as loss of sight.

Relevant to practicing dermatologists, the practical guidelines (Rex *et al*, 2000) propose strategies for the management of nongenital mucocutaneous candidiasis. Although onychomycosis is usually caused by dermatophytes (over 90% of cases), infections due to *C. albicans* species also occur (between 0%–7% to 2%). *Candida* onychomycosis responds to treatment with itraconazole (Roseeuw and de Doncker, 1993; de Doncker *et al*, 1995). Recent data from our group and others show that terbinafine has *in vitro* activity against a broad range of fungi, including yeast (Ryder *et al*, 1998; Jessup *et al*, 2000b). Non-hematogenous primary skin candidal infections are effectively treatable with topical azoles, polyenes, and allylamines. Finally, systemic antifungal therapy with azoles is efficacious for the treatment of chronic mucocutaneous candidiasis.

It is clear that *Candida* is an emerging pathogen that surfaced in the last two decades as a result of advancements in modern medicine and therapy. The epidemiology of this disease is constantly changing in response to alterations of the host immune status by, for example, HAART therapy. Moreover, the pattern of *Candida* spp. causing disease is also dynamic and influenced by the introduction of newer antifungals. These changes are impacting the way physicians manage patients with candidiasis.

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